Possible divergent reproductive strategies in New Zealand riverine non-migratory *Galaxias* fishes

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Abstract

Spawned eggs of non-diadromous *Galaxias paucispondylus* Stokell (alpine galaxias) were found in the field and genetically identified, for the first time. Eggs were found primarily in leaf pack areas of backwaters, however, the exact spawning location is uncertain and may be highly cryptic. Eggs of *G. paucispondylus*, identified genetically, were on average larger than those of *G. vulgaris*. Our findings provide evidence that supports the suggestion that pencil galaxias species, including *G. paucispondylus*, may display divergent spawning strategies from members of the *G. vulgaris* species complex. Pencil galaxias may tend to spawn in inaccessible interstitial spaces, and/or widely scatter their eggs; whereas *G. vulgaris* species complex members tend to spawn eggs in nests, or within defined areas. Possible differences in life history strategies of these two groupings of non-migratory fishes is further suggested by pencil galaxias having larger eggs and lower fecundities per unit length of fish than members of the *G. vulgaris* species complex.

Keywords: alpine galaxias - *Galaxias paucispondylus* - Canterbury galaxias - *Galaxias vulgaris* - Galaxiidae - fish eggs - spawning site - Waimakariri River.

Introduction

Life history strategies of *Galaxias* fishes occurring in New Zealand are generally considered to be either amphidromous (although some species can establish landlocked populations), or non-diadromous (McDowall 1970; 2006). Species in this latter grouping may be further differentiated based upon their provenance and morphological characteristics. One grouping termed 'pencil galaxias' (*sensu* McDowall & Waters 2003), comprises *Galaxias paucispondylus* Stokell, *G. divergens* Stokell, *G. prognathus* Stokell, *G. cobitinis* McDowall & Waters, and *G. macronasus* McDowall & Waters. The second

grouping, the *G. vulgaris* species complex (Allibone *et al.* 1996), is a specious group believed to have derived recently from successive invasions of *G. brevipinnis* Günther, with subsequent losses of diadromy (Waters & Wallis 2001). The species complex includes *G. vulgaris* Stokell, *G. anomalus* Stokell, *G. depressiceps* McDowall & Wallis, *G. eldoni* McDowall, *G. gollumoides* McDowall & Chadderton, *G. pullus* McDowall, and at least four lineages of uncertain taxonomic status. The pencil galaxias, including *G. paucispondylus* are more slender and elongate than fishes in the *G. vulgaris* species complex (McDowall & Waters 2003).

The recent recognition that *G. vulgaris sensu lato* is a complex of species, has highlighted the need to further understand the biology of riverine non-diadromous *Galaxias* species. This has led to a shift away from the notion that all these fishes share similar breeding biology. The main differences however, may be between the two non-migratory *Galaxias* groupings. Various studies have found the eggs of *G. vulgaris* species complex members deposited in large masses, either in nest-like situations or within discrete areas (Benzie 1968; Cadwallader 1976; Allibone & Townsend 1997; Moore *et al.* 1999; N.R. Dunn unpublished data). In contrast, few studies have considered the breeding biology of pencil galaxias; yet Hopkins (1971) suggested that *G. divergens* may scatter its eggs by repeated deposition of small batches, based on laboratory and field studies.

The objective of the present study was to locate *G. paucispondylus* eggs in the field, with the aim of describing their spawning location. Further, we sought to examine the hypothesis that *G. paucispondylus* has a similar spawning strategy as that suggested for other pencil galaxias and in contrast to *G. vulgaris* species complex members.

Methods

Study site

An egg survey was conducted at one of Dunn's (2003) monitoring sites on Lower Farm Stream (New Zealand Map Series 260 L34 E2412675 N5799615, altitude 537 m) in the upper Waimakariri River catchment, East Coast, South Island, New Zealand. This site contained a large *G. paucispondylus* population (mean abundance of 175 fish / 30 m of stream), which dominated the fish community. The majority (77.5%) of these were however, only 0+ and likely sexually immature at the time of the egg survey. *Galaxias vulgaris* was the only other fish species present in the stream, and then only at low abundances (7 fish / 30 m of stream). The site was located on the alluvial greywacke gravel fan in the lower reaches of the approximately 6 km^2 catchment, where the stream is of second order and has a mean instantaneous discharge of 0.12 $m^3 s^{-1}$. A mean reach slope of 0.053 mm^{-1}, median particle size (d_{50}) of 100 mm (range 0.033 – 730 mm), and a mean water temperature of 9 °C, were recorded over the period November 2001 – March 2003 (Dunn 2003). In the vicinity of the site, riparian vegetation was dominated by pasture grasses and matagouri (*Discaria toumatou*), but higher in the catchment beech (*Nothofagus* spp.) forest occurred.

Egg survey

The survey was conducted on 19 and 20 October 2002, in a 240 m reach of the stream. Two approaches were used in sampling this reach: (1) an extensive, non-

destructive search for eggs throughout the reach; and (2) intensive searches for eggs in nineteen calmer areas where visibility was high, which included both backwater areas and locations in the main channel. Backwater areas, formed by embedded boulders, contained silt, sand, and fine gravel, and at times *Nothofagus* spp. leaf packs, and other organic debris. In both extensive and intensive searches, 'turkey basters' (large bulb pipettes; d.line – Donaldson Enterprises Pty Ltd, Brisbane, Australia) were used to sample underneath boulders, within interstitial spaces, backwaters and leaf packs. Intensive searches focused on a discrete area, with larger cobbles and boulders at the heads of riffles being lifted out of the stream to penetrate deeper into the substratum. At intensively searched locations, water temperature and substratum size were recorded. Leaf pack area, where present, and backwater area and volume were also calculated.

The diameters of located eggs were measured when possible to the nearest 0.02 mm with callipers before being preserved in 95% ethanol. Of those eggs collected, a selection were genetically identified to species, using mitochondrial-DNA, following the methods of Charteris & Ritchie (2002), and Waters & Wallis (2001), by P.A. Ritchie (then Massey University, New Zealand).

Results

A total of twenty-one eggs were located in the survey reach. Of eggs successfully identified genetically, from five locations, five were *G. paucispondylus* and two were *G. vulgaris*. In one instance eggs of both species were present in the same backwater area. Eighteen eggs were measured, which ranged from 2.3 – 3.1 mm in diameter. Genetically identified *G. paucispondylus* eggs had a mean diameter of 2.7 mm (Figure 1), whereas those eggs identified as *G. vulgaris* were smaller, with a mean diameter of 2.5 mm.

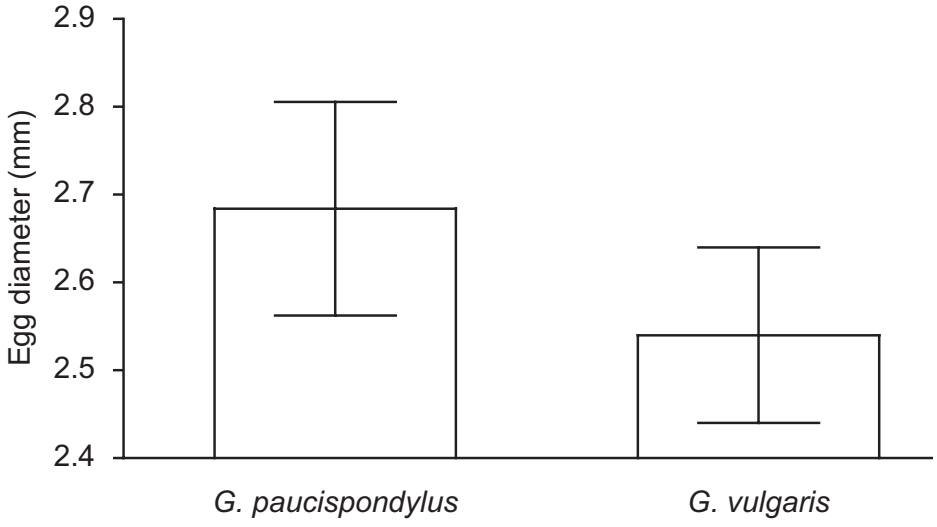

Figure 1. Mean (± 1 SEM) diameter (mm) of genetically identified *Galaxias paucispondylus* and *G. vulgaris* eggs collected from Lower Farm Stream on 19 and 20 October 2002.

Differences were not significantly different, likely due to small sample sizes, or only subtle interspecific differences, but could still be biologically important.

Eggs were found in 60% of both backwater and non-backwater locations searched (Table 1). A greater number of eggs however, were found in backwater areas. Eggs were found only in intensively searched locations and there was no evidence of nests or clumps of eggs. The majority of eggs were located singly. Only two eggs were found attached together in a non-backwater area, both of which appeared dead. The greatest number of eggs located in one area was five in a backwater area. These eggs were found buried at different depths within a backwater leaf pack (max leaf pack depth 3 cm), at its downstream end and most were alive and attached to leaf pack debris. Other locations had; no (n = 8 locations), one (n = 8), or four (n = 2) eggs.

To examine if measured backwater and leaf pack parameters could explain the presence or absence of eggs, a series of one way ANOVA analyses were conducted in Statistica 6.0 using transformed (Log_{10} [X + 1]) data. In those backwater locations with eggs, both backwater area ($F_{2,12}$ = 18.86, P < 0.001; mean ± 1 SEM with eggs = 0.71 ± 0.14 m^2, without eggs = 0.26 ± 0.17 m^2), and leaf pack area ($F_{2,12}$ = 4.59, P = 0.033; mean ± 1 SEM with eggs = 0.04 ± 0.01 m^2, without eggs = 0.01 ± 0.02 m^2), were found to be significantly larger than those backwater locations without eggs.

Discussion

Due to constraints on energy acquisition and body volume available to the gonads in fishes, there is a strong tendency for a trade-off between egg size and fecundity (Blaxter 1969). Differences in the solution to this trade-off form the basis of life-history and reproductive variations seen in fishes (McDowall 1970). Thus, relative egg size and female fecundity can give an indication of likely successful spawning strategies. It is usually considered that fish producing many, small eggs can compensate for high initial offspring

Table 1. Summary of egg survey results detailing numbers of eggs found in backwaters, and non-backwater locations, numbers dead or alive and materials to which eggs were found adhered too.

	Backwaters	**Non-backwaters**
Without eggs	6	2
With eggs	8	3
Number of eggs		
alive	12	3
dead	3	3
Number attached to:		
debris	3	1
sediment	3	0
Nothofagus spp. leaf	2	0

mortality; whereas, species that produce fewer, larger eggs, require higher survival of early life stages. Larger egg size represents greater individual oocyte investment and results in larger larvae with greater initial survival (Bagenal 1969), but this may mean they are more conspicuous to predators. Eggs are one of the most vulnerable life stages and various strategies for ensuring high egg survival have been observed in galaxiids, which are known to cannibalise eggs (Allibone 2003; O'Brien 2005). Galaxiid fishes may be able to increase egg survival either through parental care (Cadwallader 1976), placement of eggs in situations inaccessible to predators (Eldon 1971; McDowall & Charteris 2006), and/or by scattering eggs to increase the chances of eggs being overlooked by predators (O'Brien 2005; O'Brien & Dunn 2005).

Genetically identified *G. paucispondylus* eggs collected from Lower Farm Stream were on average larger than *G. vulgaris* eggs. Thus, our findings support Bonnett (1992), in that *G. paucispondylus* produce a small number of large eggs. Furthermore, un-spawned oocytes of other pencil galaxiids appear to be similar in size (1.8 – 2.2 mm diameter; Hopkins 1971; Bonnett 1992; L.K. O'Brien & N.R. Dunn unpublished data). Although direct comparisons between un-spawned and spawned eggs are problematic because eggs expand in water, spawned egg sizes are similar within the *G. vulgaris* species complex, e.g. *G. anomalus* (mean ± 1 SEM: 2.3 ± 0.02 mm), and *G. depressiceps* (2.5 ± 0.01 mm; Allibone & Townsend 1997). Related and more compelling differences between non-migratory galaxiid groups occur for fecundity, whereby pencil galaxiids, especially *G. paucispondylus*, have lower fecundity than members of the *G. vulgaris* species complex (Table 2). Although, female fecundity can vary within a species, and between years and habitats (Allibone & Townsend 1997), the differences in fecundity between pencil galaxias and *G. vulgaris* complex species are substantial and are likely to be biologically significant. Thus, solutions to the trade-off between egg size and fecundity indicate possible differences between these galaxiid groups, with *G. paucispondylus* and possibly other pencil galaxiids considered to have fewer, larger spawned eggs than *G. vulgaris* species complex members.

It is difficult to determine from the survey results whether the scattered eggs found were 'strays' swept down from an upstream concentrated spawning site or were deposited directly in leaf packs. Considering that *G. vulgaris* eggs, usually deposited in nests, were found in the same backwater area as *G. paucispondylus* eggs and that larger backwater depositional areas were more likely to contain eggs, it seems likely that those eggs found were strays. However, this does not discount the possibility that in some cases eggs had been spawned in small batches in backwaters. Spawned galaxiid eggs are initially very adhesive, but this property is reduced as they accumulate microfilms and fine debris (O'Brien 2005). A greater proportion of eggs were found alive and attached to surrounding substrata in backwaters, supporting the suggestion that eggs were spawned within the backwaters themselves. Moreover, eggs were found in backwaters in which adult fish had been observed occupying during the previous evening. However, despite thorough searches, this study did not conclusively find the spawning location of

Table 2. Comparison of the number of eggs per millimetre length of fish for pencil galaxias' and members of the *Galaxias vulgaris* species complex. Figures are derived from available information, where possible fecundity – fish length equations have been used, based on a theoretical 100 mm total length females, otherwise mean values (*) are given.

Species	Number of eggs / mm of fish	Source
Pencil galaxias		
G. paucispondylus	1.6	Bonnett (1992)
G. prognathus	2.6	Bonnett (1992)
G. cobitinis*	2.0	O'Brien& Dunn (unpublished data)
G. divergens	2.6	Hopkins (1971)
G. macronasus*	2.9	McDowall & Waters (2003)
G. vulgaris species complex		
G. vulgaris	14.5	Cadwallader (1976)
G. anomalus	8.7	Allibone & Townsend (1997)
G. depressiceps	5.0	Allibone & Townsend (1997)
G. eldoni*	5.2	Allibone & Townsend (1997)

G. paucispondylus, as would be indicated by finding numerous eggs, which suggests a cryptic spawning location. Low fecundity and relatively large egg size, which would necessitate high egg survival, also indicates that a successful spawning strategy for *G. paucispondylus* may involve either widely scattering their eggs, as suggested for *G. divergens* (Hopkins 1971), and/or deposition of eggs in inaccessible situations. Recently the eggs of *G. cobitinis* have been found deep within the interstitial spaces of gravel bed substratum in an area of water upwelling (P.J. Ravenscroft & D.C. Jack, Department of Conservation, Dunedin, pers. comm.). It is possible that other pencil galaxiids, including *G. paucispondylus*, also utilise similar spawning locations, especially as they are capable of gravel burrowing (Hartman 1990; Dunn 2003; Dunn & O'Brien 2006).

In conclusion, this study found scattered spawned eggs in the field that were genetically identified as *G. paucispondylus*, and were determined to be relatively large. These findings suggest that not only are pencil galaxiids morphologically different from other non-diadromous *Galaxias* species, but that further differences in reproductive behaviour and life-history strategy possibly exist.

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